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Epidemic status of *Angiostrongylus cantonensis* in Hainan island, ChinaXimin Hu<sup>\*</sup>, Jianwei Du<sup>\*</sup>, Chongjin Tong, Shanqing Wang, Jian Liu, Yuchun Li, Changhua He

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## ABSTRACT

**Objective:** To understand the current epidemiological status of *Angiostrongylus cantonensis* in Hainan island and provide the scientific evidences for prevention and control of the disease. **Methods:** The investigation on nature infection condition of intermediate hosts and final hosts of *Angiostrongylus cantonensis* were carried out in five counties, Hainan Province, and enzyme linked immunosorbent assay (ELISA) was employed to detect antibodies of *Angiostrongylus cantonensis* in human hosts. **Results:** A total of 1 612 molluscs were examined, 21.3% of which harbored L3 of *Angiostrongylus cantonensis*. Among them, the infection rates of *Pomacea canaliculata*, *Achatina fulica*, *Cepaea* and *Phlegm bilineatus* were 12.36%(64/518), 22.66%(121/534), 20.93%(9/43), and 28.24%(146/517), respectively. Of the 118 rats trapped, 13 *Rattus norvegicus* were found to be infected with *Angiostrongylus cantonensis*. A total of 459 serum samples were collected and tested. 92 serum samples were *Angiostrongylus cantonensis* antibody-positive. **Conclusions:** The survey revealed a wide distribution of *Angiostrongylus cantonensis* in Hainan island. *Pomacea canaliculata* and *Achatina fulica* are main intermediate hosts of *Angiostrongylus cantonensis*. *Rattus norvegicus* is a nature definitive host, indicating that a considerable number of people are at risk of angiostrongyliasis. Health education, rigorous food inspection and surveillance are all needed to prevent angiostrongyliasis outbreaks in future.

## 1. Introduction

The nematode worm *Angiostrongylus cantonensis*(*A. cantonensis*) was discovered in the pulmonary arteries and hearts of domestic rats in Guangzhou (Canton), China, by Chen in 1935[1]. *A. cantonensis* is a zoonotic pathogen, which occasionally causes human angiostrongyliasis with the main clinical manifestation of eosinophilic meningitis. The first human infection case of angiostrongyliasis was reported in Taiwan in 1945[2]. Since then, several outbreaks of the disease in human beings have been reported in the Pacific islands[3]. In the past 10 years, several major outbreaks of the disease have been reported in endemic regions, especially in China, causing great concern for both the

general public and physicians[4].

Hainan island is located in the southern end of China, 18°10'–20°10'N latitude and 108°37'–111°03'E longitude, a tropical zone. The climate is warm with plentiful rainfalls, proper humidity, and it is quite suitable for survival and transmission of *A. cantonensis*. Hainan island as one of a province in China including 18 cities and counties, Xisha archipelago, Zhongsha archipelago and Nansha archipelago with a population of 8.28 million and total areas of 35 400 square kilometers. To understand the distribution, infectious status of *A. cantonensis* and its hosts in the geographic region, a survey on the distribution, infectious status of *A. cantonensis* and its intermediate hosts in Hainan island was carried out from 2007 to 2009. Here we reported significant findings.

## 2. Materials and methods

## 2.1. Survey sites selection

According to different geographic locations with the

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distribution of water systems, five counties or cities in Hainan island were selected as the survey sites. The five counties or cities are Haikou, Sanya, Danzhou, Qionghai, Qiongzong, respectively. In each survey sites, one village was randomly selected for subsequent field work.

## 2.2. Host etiology examination

Rats were trapped in fields and in residents' houses. All captured animals were euthanized and dissected to determine the presence of adult *A. cantonensis* in their hearts and lung arteries. Freshwater snails, terrestrial snails and certain terrestrial slugs were collected from the surroundings of the study villages, and from restaurants and markets in the capital town of the counties, which were examined for the presence of *A. cantonensis* larvae. Up to 100 specimens of each species were collected at each study site. The intermediate hosts were artificially digested using routine procedures[5]. Additionally, for the examination of *Pomacea canaliculata* (*P. canaliculata*), a recently developed method relying on specific lung tissue features of this species was employed[5,6]. In brief, the lungs were separated from the snail body and opened for checking the parasites. The nodules containing *A. cantonensis* larvae were then directly observed under a microscope. Paratenic hosts were also collected from markets and restaurants for examination of L3 using an artificial digestion method.

## 2.3. Seroepidemiological survey

Collecting serum from permanent residents in five survey sites, ELISA was employed to detect the antibodies IgG against *A. cantonensis* in serum (Test kit producer: Shenzhen Bombined Biotech Co. Ltd, China ).

## 3. Results

### 3.1. *A. cantonensis* hosts and their infection status

Of the 118 rats trapped during the field surveys, 13 *Rattus norvegicus* were found infected with *A. cantonensis*. The infection rate was 11.02%. A total of 1 612 molluscs were examined, 21.3% of which harbored L3 of *A. cantonensis*. Among them, the infection rates of *P. canaliculata*, *Achatina fulica* (*A. fulica*), *Cepaea* and *Phlegm bilineatus* were 12.36%(64/518), 22.66%(121/534), 20.93%(9/43), 28.24%(146/517), respectively.

### 3.2. Intermediate host snails from markets and restaurants

A total of 680 snails collected from markets and/or restaurants in five survey sites were examined. The overall infection rate was 10.29% (41/680). Among them, the infection rates of *P. canaliculata* was 9.67%(41/424) and *A.*

*fulica* was 28.43%(29/102). Infected *P. canaliculata* and *A. fulica* were found in four and one counties, respectively. Additionally, one native freshwater snail species, *Cipangopaludina chinensis* was commonly found sale in many markets and none of them were checked positive.

## 3.3. Serological test results

A total of 459 serum samples were collected and tested, and 92 serum samples were *A. cantonensis* antibody-positive. Overall antibody positive rate was 20.04%, Antibody-positive rate in males and female were 18.62% (35/188) and 20.36% (57/280), respectively, without significant gender differences ( $\chi^2 = 0.22, P > 0.05$ ). Antibody positive rate of people under the age of 14 (including 14 years of age) was 29.23% (19/65), and over the age of 14 was 18.53% (73/394), with significant age difference ( $\chi^2 = 3.99, P < 0.05$ ).

## 4. Discussion

Eosinophilic meningitis caused by *A. cantonensis* is endemic in Southeast Asia, Australia, the Pacific islands and the Caribbean. To date, more than 2 800 human cases have been reported. It had been suggested that the parasite was dispersed from East Asia to other regions in two important hosts, *i.e.*, rats (definitive host) and *A. fulica* (intermediate host) especially during World War II[7]. Today, the parasite is still expanding and the associated disease is emerging in some regions, particularly in China. Hainan Province is located in the southern end of China, a tropical zone. The climate is warm with plentiful rainfalls and proper humidity, and it is quite suitable for the transmission of *A. cantonensis*. As early as in 1984, a small-scale survey on the infectious status of intermediate hosts of *A. cantonensis* had been carried out in Wenchang County, Hainan Province[8]. The survey results showed that the infection rates of *A. fulica* were 33.3% (10/30). The results of the first provincial survey on the distribution of *A. cantonensis* and its hosts in Hainan Province indicate that the *A. cantonensis*-endemic area is very broad, covering all the counties surveyed. *P. canaliculata* and *A. fulica* are the primary intermediate hosts, *Rattus norvegicus* is the major definitive host in Hainan Province. However, no angiostrongyliasis cases have been observed thus far in Hainan Province. The important indicators might have been neglected or misdiagnosis due to low levels of awareness about angiostrongyliasis among medical staff. Many cases could have gone with unreported or unrecognized during the past decades. It remains to be further investigated. Hospital-based surveillance might be an effective approach to reveal unfolding angiostrongyliasis epidemics in areas where snails, particularly *P. canaliculata* and *A. fulica*, are popular.

*A. fulica* was recorded for the first time in mainland China

in 1931[9]. It has been suggested that eggs of *A. fulica* were accidentally imported from Singapore with shipments of plants, and that an initial snail population became established in Xiamen (Amoy)[10]. These terrestrial snails are nocturnal and become active under high-humidity conditions. The snails feed on plants and deposit their eggs in the soil nearby. Subsequently, the snail was farmed in most southern provinces with commercial aims[11]. It is conceivable that the dense river networks in Hainan island contributed to the dispersal of this snail. *A. cantonensis* infection is mediated not only by ingestion of the *A. cantonensis* –carrier intermediate or paratenic hosts but also through the ingestion of vegetables, drinking water, and by contact with fingers that are contaminated by the infective larvae of *A. cantonensis*. Small planarians could represent a very important but overlooked source of human infection when they are consumed together with contaminated uncooked vegetables.

The results of the survey also suggest that people are at risk of angiostrongyliasis through consumption of raw or undercooked snails infected with *A. cantonensis* in some markets and restaurants. It is important to enhance the understanding of angiostrongyliasis epidemiology, increase public awareness about the risks associated with eating raw food, and enhance food safety measures. Food safety and transportation must be improved to avoid human infections and the further spread of intermediate host snails to areas in which the disease is not endemic. Collection and marketing of wild snails should be limited. Hygiene and food preparation techniques in restaurants should be improved to prevent from cross-contamination of other food items. These efforts should be accompanied by sound information in communication and education campaigns to raise public awareness. The basic message, that consumption of raw or undercooked snails is a key risk factor for the transmission of a serious disease, can be easily conveyed and is readily understood by the public. Awareness of angiostrongyliasis needs to be improved for consumers and health professionals. Education campaigns should inform consumers about the risk of contracting angiostrongyliasis, e.g., by eating raw snails and professional knowledge among healthcare providers should be improved to ensure timely detection of infections and adequate medical response. Travellers heading to endemic regions must be made aware of the dangers of eating raw molluscs and vegetables from unknown sources and should avoid these foods. Washing hands frequently, particularly after gardening, is also strongly emphasised. Overall, Continued health education,

rigorous food inspection, and hospital-based surveillance are needed to prevent from potential angiostrongyliasis outbreaks in Hainan island.

### Conflict of interest statement

We declare that we have no conflict of interest.

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